Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients

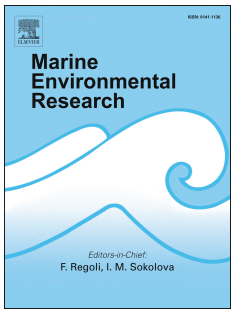
Sampaio, E.

2017-04

Sampaio , E , Franco Rodil , I , Vaz-Pinto , F , Fernandez , A & Arenas , F 2017 , ' Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients ' , Marine Environmental Research , vol. 125 , pp. 25-33 . https://doi.org/10.1016/j.marenvres.2017.01.001

http://hdl.handle.net/10138/309461
https://doi.org/10.1016/j.marenvres.2017.01.001

Accepted Manuscript



Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients

E. Sampaio, I.F. Rodil, F. Vaz-Pinto, A. Fernández, F. Arenas

PII: S0141-1136(16)30219-7

DOI: 10.1016/j.marenvres.2017.01.001

Reference: MERE 4262

To appear in: *Marine Environmental Research*

Received Date: 19 October 2016

Revised Date: 27 December 2016

Accepted Date: 5 January 2017

Please cite this article as: Sampaio, E., Rodil, I.F., Vaz-Pinto, F., Fernández, A., Arenas, F., Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients, *Marine Environmental Research* (2017), doi: 10.1016/j.marenvres.2017.01.001.

Interaction strength between different grazers and macroalgae mediated by ocean acidification over warming gradients.

Macroalgae-herbivore interactions in changing oceans

E. Sampaio[1,2]*, I. F. Rodil[1,3,4], F. Vaz-Pinto[1], A. Fernández[1], F. Arenas[1]

[1]Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR) – University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

[2]MARE - Marine Environmental Science Centre, Laboratório Marítimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Av. Nossa Senhora do Cabo 939, Cascais 2750-374, Portugal

[3]Tvärminne Zoological Station, University of Helsinki, Hanko, Finland

[4]Baltic Sea Centre, Stockholm, University, Stockholm, Sweden

***corresponding author:** edusilvasampaio@gmail.com

Abstract

Since the past century, rising CO_2 levels have led to global changes (ocean warming and acidification) with subsequent effects on marine ecosystems and organisms. Macroalgae-herbivore interactions have a main role in the regulation of marine community structure (top-down control). Gradients of warming prompt complex non-linear effects on organism metabolism, cascading into altered trophic interactions and community dynamics. However, not much is known on how will acidification and grazer assemblage composition shape these effects. Within this context, we aimed to assess the combined effects of warming gradients and acidification on macroalgae-herbivore interactions, using three cosmopolitan species, abundant in the Iberian Peninsula and closely associated in nature: the amphipod *Melita palmata*, the gastropod *Gibbula umbilicalis*, and the green macroalga *Ulva rigida*. Under two CO_2 treatments ($\Delta CO_2 \simeq 450$ µatm) across a temperature gradient (13.5, 16.6, 19.9 and 22.1 ºC), two mesocosm experiments were performed to assess grazer consumption rates and macroalgae-herbivore interaction, respectively. Warming (Experiment I and II) and acidification (Experiment II) prompted negative effects in grazer's survival and species-specific differences in consumption rates. *M. palmata* was shown to be the stronger grazer per biomass (but not per capita), and also the most affected by climate stressors. Macroalgae-herbivore interaction strength was markedly shaped by the temperature gradient, while simultaneous acidification lowered thermal optimal threshold. In the near future, warming and acidification are likely to strengthen top-down control, but further increases in disturbances may lead to bottom-up regulated communities. Finally, our results suggest that grazer assemblage composition may modulate future macroalgae-herbivore interactions.

45 Key-words: warming, acidification, macroalgae-herbivore interaction, stressor gradient,

46 interaction strength.

Introduction

Anthropogenic CO_2 accumulates in the atmosphere, retaining infrared radiation and rising temperatures of both terrestrial and ocean ecosystems. As a result, global average sea surface temperature increased approximately 0.1 °C per decade during the last 40 years (Taboada and Anadón, 2012) and a further 2-5 ºC increase in mean sea surface temperature is predicted by 2100 (IPCC, 2013). Biologically, temperature affects metabolic rates by altering biochemical reactions' kinetics and energies. According to basic metabolic theory, all organisms possess a survival thermal window, where increasing temperature increases reaction rates until an optimal level is reached (Angilletta, 2009). Beyond this threshold, further increases in temperature cause physiological stress (e.g. protein denaturation), resulting in a steep decline in metabolic rates and consequently in biological processes (see Fig. 1 in Kingsolver, 2009) such as growth, development and feeding activity (Angilletta, 2009; Kingsolver, 2009; Mertens et al., 2015). In fact, due to differential effects on autotrophic and heterotrophic metabolisms, metabolic theory of ecology (MTE) dictates that producers will show a weaker response to temperature than consumers (Yvon-Durocher et al., 2010). Thus, as individual changes lead to community effects (Mertens et al., 2015), small increases in temperature are predicted to strengthen top-down control by herbivores on marine primary producers, as a consequence of higher herbivore consumption rates relative to macroalgal production (Eklöf et al., 2012; O'Connor, 2009). Furthermore, it is also possible that marine communities find an equilibrium by balancing herbivore grazing and algal biomass growth, through compensatory mechanisms which allow for community stabilization (Connell and Ghedini, 2015).

The dissolution of atmospheric CO_2 into the oceans alters seawater carbonate system and the associated drop in pH levels is known as ocean acidification. Since the past

century, atmospheric CO_2 concentrations increased to approximately 400 µatm and are predicted to reach 1000 µatm by the end of this century, with a corresponding drop of 0.14-0.35 units in mean ocean pH (IPCC, 2013). Calcifying animals and algae are deemed as the most imperiled marine organisms due to deregulation of acid-base processes, decrease in calcification rates and/or increase in calcium carbonate dissolution, and hypercapnic growth inhibition (Byrne, 2011; Koch et al., 2013; Kroeker et al., 2011). Non-calcifying organisms register quite different responses across taxa (Connell and Russell, 2010; Kroeker et al., 2010; Poore et al., 2013), since some species of crustaceans and fish show aptitude to partially offset the negative effects of acidification through mechanisms of acid-base compensation, changes in metabolism, energy reallocation and/or increase in mobility (Larsen et al., 1997; Melzner et al., 2009; Widdicombe and Spicer, 2008; Wood et al., 2008). For seaweeds, dissolved CO_2 is normally used as a substrate for photosynthesis, and non-calcareous species may benefit from increases of this resource (Koch et al., 2013). Nevertheless, some species, e.g. *Ulva rigida*, are unaffected by increased CO_2, as present day carbon concentrations already saturate its photosynthetic and growth physiological processes (Rautenberger et al., 2015). Identically to warming effects, ocean acidification may affect species interaction strength due to differential sensitivities to pH changes, potentially cascading into alterations in species competition dynamics and trophic interactions (Falkenberg et al., 2013; Hepburn et al., 2011; Kroeker et al., 2012).

To date, most research performed examining climate change impacts of marine systems have used multi-level single stressor (Connell et al., 2013; Falkenberg et al., 2013; O'Connor, 2009), straightforward two-stressors/two-levels (2 x 2) (Connell and Russell, 2010; Gaitán-Espitia et al., 2014; Veteli et al., 2002), or single species experimental designs (see Wernberg et al., 2012 for a review). However, climate change

will create new complex physicochemical scenarios characterized by multi-levels of stressors interacting. It is critical to improve our ecological knowledge on the potential combined effects of global stressors not only on different trophic levels, but also on interacting communities to strengthen our capacity in predicting future impacts of climate change on marine ecosystems (Poore et al., 2013). Whilst an increase in top-down control is likely under warming alone (López-Urrutia et al., 2006; O'Connor, 2009), forecasting impacts on macroalgae-herbivore interactions elicited by temperature and acidification effects is far more complex, given the non-linear variation in organism metabolic rates across stressor gradients (Mertens et al., 2015). Further increasing temperature (Mertens et al., 2015) and/or exposure to multiple stressors (Ghedini et al., 2015a) are reported to overcome the compensatory/grazing metabolic threshold, leading to shifts in community regimes, from top-down (herbivore-regulated) to bottom-up (algae- or nutrient-regulated) systems. Moreover, studies comparing mono-specific herbivore populations *versus* mixed herbivore populations under climate change scenarios are scarce (Alsterberg et al., 2013), but essential to understand community effects on natural systems. Within this context, here we studied how distinct CO_2 concentrations across a temperature gradient would differently impact the survival and consumption rates of two common herbivore species from the rocky intertidal; the non-calcifying amphipod species *Melita palmata* (Montagu, 1804), and the calcifying gastropod species *Gibbula umbilicalis* (da Costa, 1778). Additionally, we tested how these climate stressors would alter algal biomass of an abundant rocky intertidal algal species, *Ulva rigida* (Agardh, 1823). Finally, we hypothesized that community dynamics, i.e. macroalgae-herbivore interaction strength, would be interactively modified by temperature and CO_2. Simultaneously, we investigated if grazer species

identity may also play a role in shaping community response, due to differential effects on grazers' survival and consumption rates.

Methods

Study species

Gastropods are considered efficient grazers (Jernakoff and Nielsen, 1997), generally grazing larger algal portions than amphipods (Morrisey, 1988). We used the calcifying gastropod *Gibbula umbilicalis* as a large grazer species and the non-calcifying amphipod *Melita palmata* as a small-bodied grazer (~1/10 biomass of *G. umbilicalis*) to assess the joint effects of different climate change stressors in macroalgae-herbivore interactions. Both macroinvertebrate species are common grazers from the western Atlantic intertidal rocky coast of the Iberian Peninsula. They can be found associated to mixed macroalgal beds, including large accumulations of *Ulva spp.*, searching for food and refuge during low intertidal. The green seaweeds from the *Ulva* genus are very common opportunistic primary producer in shallow coastal waters and tidal pools in rocky shore communities (Aníbal et al., 2007).

The three species (i.e. *G. umbilicalis*, *M. palmata* and *U. rigida*) were collected on the same date at Viana do Castelo (North of Portugal, 41°41'44"N, 8°51'2"W), and were later separated by hand in the laboratory. To select our experimental gradient of temperatures, we registered water temperature from the sampling location through two continuous temperature data loggers (Tidbit V2 Onset HOBO®) installed at two mid-shore rockpools, during the month of August, 2013 (date of this study). Daily average temperature in the seawater of these rockpools ranged from 14.96 ± 0.72 ºC to 17.80 ± 2.11 ºC (mean ± SD) depending on night/day phase, with 13.28 ºC and 25.23 ºC recorded as the absolute minimum and maximum values, respectively. This large

gradient of temperatures in rock pools from this region results from a combination of frequent summer upwelling events (Lemos and Pires, 2004), and prolonged exposure to the sun without water exchange during low tides. As we were interested in investigating realistic interactions between pH and temperature, CO_2 concentrations followed the "business-as-usual" predicted scenario for 2100 (IPCC, 2013) and mean experimental temperatures were chosen within the thermal limits of *G. umbilicalis* (Evans 1948), *U. rigida* (Steffensen 1976) and *M. palmata* (Obenat et al. 2006). We used adult individuals of amphipods (0.96 ± 0.19 cm, mean length measured from the tip of the head to the telson) and gastropods (1.05 ± 0.18 cm, mean length measured from the tip of the operculum to the furthest point in the horizontal plane).

Experimental mesocosm setting

Experimental mesocosm (closed system) consisted of eight transparent PVC tanks (approx. 100 L, 50 x 50 x 40 cm, hereafter referred as treatment tanks) filled with seawater and subjected to eight crossed treatment combinations of two CO_2 levels: ~380 ± 30 µatm (ambient) and ~830 ± 45 µatm (increased), and four temperature levels (mean ± SD, gradient): 13.5 ± 2.0 ºC (low), 16.1 ± 2.0 ºC (midlow), 19.9 ± 3.0 ºC (midhigh), and 22.1 ± 2.0 ºC (high) (see Figure S1). As mean rock pool temperature was approximately 16 ºC (see also Cacabelos et al. 2013 for confirmation of similar values), midlow treatment was acknowledged as mean natural temperature conditions. Temperature was controlled through titanium aquarium heaters, maintained and adjusted by AT Control devices (Aqua Medic®). Seawater pH was maintained by manipulating seawater carbonate chemistry, constantly monitored and registered with Aqua Medic pH electrodes. Each treatment tank (n = 8) connected to a separated PVC header tank (100 L of capacity, n = 8), where the incorporation of enriched CO_2 /

normal air was performed. To adjust pH, a pH-stat system (Aqua Medic®, AT Control) injected certified CO_2-enriched air (Air Liquide) through solenoid valves to down regulate pH, and normal air to up regulate pH. Seawater carbonate chemistry was calculated based on, temperature, salinity, total alkalinity (assessed spectrophotometrically at 595 nm) and pH_t measurements (Sarazin et al., 1999) (Table S1). As such, pH_t was quantified through a Metrohm pH meter (826 pH mobile, Metrohm, Filderstadt, Germany) connected to a glass electrode (Schott IoLine, SI analytics, ± 0.001), which was calibrated with 2-aminopyridine-HCl (AMP) and TRIS-HCl (TRIS) seawater buffers following Dickson et al. (2007). Total carbonate and pCO_2 (Table S1) were calculated using CO2SYS software (Lewis and Wallace, 1998), with equilibrium constants (Mehrbach et al., 1973) refitted by Dickson and Millero (1987). Abiotic conditions between header and treatment tanks were secured using submersible pumps (570 l h^{-1}), with water permanently exchanged to maintain pH treatments, using one submersible pump (3000 l h^{-1}) and gravitational force. Within each treatment tank, water exchange was enhanced through water tubing (smaller submersible pump 570 l h^{-1}) that assured all abiotic variable conditions were similar between cylinders.

Each treatment tank contained mechanical and biological filtering, as well as twelve transparent cylinders (D 0.33 x H 0.10 m, ~2 l, 0.085 m^2 cylinder base area), i.e. experimental units, consisting of four different grazer levels (G: *Gibbula umbilicalis*, M: *Melita palmata*, G+M: both species together, and C: controls with no grazers, n = 3) interspersed through the treatment tank (Figure S1). The top of each cylinder was covered with a 1.0 mm mesh to avoid animals from escaping, while still enabling water circulation and light entry. Nutrients (1 ml of 42.50 g l^{-1} $NaNO_3$ solution and 1 ml of 10.75 g l^{-1} Na_2HPO_4 solution per liter of seawater) were added to each tank every two days, and water was renewed manually when salinity increased to 36 (35.5 ± 1.0 PSU;

196 mean ± SD, n = 8). Temperature, pH and salinity were monitored three times a day with
197 the use of a data-logger. The experimental setup was placed inside a shadow greenhouse
198 under natural light and photoperiod, with consequently reduced incident light inside,
199 mimicking commonly found subsaturating light conditions on rocky pools (Guidone et
200 al., 2012; O'Connor, 2009).
201
202 *Experiment I: Warming and acidification effects on grazers consumption rates*
203 We performed a two-day (i.e. 48h) experiment to assess grazer consumption rates for
204 mono-specific and mixed grazer combinations (O'Connor, 2009). This experiment was
205 preceded by a 24h algae-free period for grazer acclimatization (algae were acclimated in
206 separate cylinders) to avoid the influence of past diet and ensure algae consumption
207 during the experiment (Swanson & Fox, 2007). We used 3-replicated cylinders with
208 four levels of grazers (density in cylinder = 0.009 ind / m^2): *G. umbilicalis* (hereafter G,
209 n = 10), *M. palmata* (hereafter M, n = 10), both species together (hereafter G+M, n = 5
210 + 5), and control (hereafter C, no grazers). At the end of the 24 hour acclimatization
211 period, algae were spun 25 revolutions in a salad spinner to remove excess water and
212 ~3.015 ± 0.041 g (mean ± SD, density in cylinder = 0.028 g algae / m^2) of algal blotted
213 wet weight (bww) were inserted in each cylinder. While in the cylinders, algae were
214 strapped to weights and left swinging inside the cylinder, mimicking natural rock pool
215 conditions. After the 48 hour grazing trial, portions were removed, blotted and weighted
216 again as above. Dead animals were recorded at the end of the experiment.
217
218 *Experiment II: Warming and acidification effects on interaction strength*
219 We performed a longer (seven-day or 168 hours) experiment with new animals and
220 algal portions to assess differences in per capita macroalgae-herbivore interaction

strength, i.e. individual grazer pressure per algal biomass growth ratio. A 24h period of acclimatization with algae was performed (Eklöf et al., 2015), and at the beginning of the experiment, algae were added, mimicking natural conditions where seaweed is abundant (Cacabelos et al., 2013; O'Connor, 2009). Three-replicated cylinders were used, with 6 grazers for each level of grazer treatment (density in cylinder = 0.014 ind / m^2): G (n = 6), M (n = 6), G+M (n = 3 + 3) and control (C, no grazers). Grazer numbers were reduced to avoid excessive competition for habitat and food. At the beginning of the experiment, ~7.030 ± 0.025 g (mean ± SD, density in cylinder = 0.012 g algae / m^2) of blotted wet weight portions of algae were strapped to weights and inserted in each cylinder. After seven days, algae were retrieved, blotted and weighted. The number of dead animals was recorded at the end of the experiment.

Data Analysis

Survival rates in both experiments were assessed according to the number of living *G. umbilicalis* and *M. palmata* individuals (and the sum of both in the case of G+M treatment) present at the end of each experiment, and transformed into survival rate (%) per cylinder. Changes in algal biomass were estimated through the difference of initial and final values of algal wet biomass in experiments. Consumption rates in Experiment I was calculated using the following formula based on Taylor and Brown (2006):

$$\text{Consumption} = (T_i * (C_f / C_i) - T_f) / (n_{bio} * t),$$

Where T_i is the initial algal bww, T_f is the final algal bww, C_i is the initial control algal bww, C_f is the final control algal bww, n_{bio} is the added biomass of living grazers in each cylinder at the end of the experiment and t is the time elapsed in the experiment

(2 days). Thus, consumption rates are expressed in grams of algae consumed per invertebrate biomass per day. Biomass was extrapolated from a referenced length-weight regression based on *M. palmata* individuals' total length (Grilo et al., 2009; Pardal et al., 2002). Likewise, *G. umbilicalis* biomass was extrapolated by measuring the longest vertical axis and fitted in a referenced length-weight regression (Robinson et al., 2010).

In Experiment II, in addition to survival rates and changes in algal biomass, alterations in community dynamics, i.e. grazing pressure and simultaneous macroalgal growth, were also analyzed. To assess the strength of macroalgae-herbivore interaction we used the Dynamic Index (Berlow et al., 1999; Mertens et al., 2015; O'Connor, 2009; Wootton and Emmerson, 2005) modified from Wootton (1997), with the following formula:

$$DI = \ln (N / D) / (n * t),$$

where DI is the Dynamic Index, N is algal wet biomass with grazers, D is the algal wet biomass on treatment without grazers, n is the number of living grazers in the cylinder, and t is the known period of time elapsed (i.e. seven days). This index measures interaction strength, i.e. the absolute value of daily per capita interaction strength, accounting for differences in algal growth rates with and without herbivores (O'Connor 2009). Negative interaction strength values implicate that one species reduces the abundance of the other species; therefore, lower values indicate stronger interactions (i.e. high grazer pressure). This index translates survival rates and metabolic alterations provoked by climate change stressors into an ecological response (O'Connor 2009). Compared to other methods, DI does not assume equilibrium between algae and

grazers, which is convenient for relatively short experiments as ours (Berlow et al., 1999).

Changes in survival rates, algal biomass, consumption rates and DI were analyzed separately through a distance-based permutational multivariate analysis of variance (PERMANOVA, Anderson 2001). We calculated distance resemblance matrices using Euclidean dissimilarity measures based on untransformed data. PERMANOVA was run with 4999 permutations to obtain *p* values under unrestricted permutation of raw data (Anderson 2001), using CO_2 (2 levels; ambient and increased), temperature (4 levels: low, midlow, midhigh and high) and grazers (4 levels: G, M, G+M and C) as fixed orthogonal factors (n = 3). The permutational approach was used because the data were non-normally distributed even after transformations (Wernberg et al., 2008). Only significant effects ($p < 0.05$) were further investigated through a series of pair-wise comparisons using the appropriate terms in the model (Anderson et al. 2008). All the multivariate analyses were performed with PRIMER 6 & PERMANOVA+ package.

Results

Experiment I: Warming and acidification on grazer consumption rates

After 48h, survival rates differed significantly depending on temperature and grazer treatments (Table 1). Specifically, survival rates decreased from lower (13.5 ºC and 16.1 ºC) to higher (19.9 ºC and 22.1 ºC) temperatures ($p < 0.001$, Figure 1, see Table S2 for pair-wise comparisons). Concerning grazer treatments, survival rates were comparatively higher in G (90%), and lower in M (~40%), while G+M registered intermediate survival rates (~70 %) (Figure 1, Table S2).

295 Algal biomass was significantly altered by temperature and the interaction between
296 CO_2 and grazers (Table 1, Figure 2). Thus, at high temperature (22.1 ºC) algal biomass
297 loss was reduced compared to biomass loss reported for the two lower temperature
298 levels, 13.5 ºC and 16.1 º C (Figure 2a, Table S3). G registered higher biomass loss then
299 M, and increased CO_2 amplified these effects (Figure 2b, Table S3). In grazer control
300 treatments, i.e. C, algal biomass increased: 0.1 g (from initial 3 g) per day ≃ 3 % per
301 day.

302 Consumption rates were significantly affected by the triple interaction between CO_2,
303 temperature and grazer (Table 1). Multiple pairwise comparisons revealed that
304 consumption rates in M were always higher than in G and G+M, regardless of
305 temperature or CO_2 (Figure 3, Table S4). Comparing CO_2 treatments, at 13.5 ºC, G+M
306 displayed lower consumption rates in increased CO_2 (Figure 3, Table S4). Under
307 ambient CO_2 (Figure 3a), G and G+M consumption rates dropped across the
308 temperature gradient, whereas M consumption rates increased (Figure 3, Table S4).
309 Lastly, under increased CO_2, temperature did not different consumption rates in any
310 grazer treatment (Figure 3b, Table S4).

311

312 *Experiment II: Warming and acidification effects on interaction strength*

313

314 After 7 days (i.e. 168h), survival rates were significantly affected by variations in
315 CO_2 and the interaction between temperature and grazers (Table 2). Lower survival
316 rates were reported under increased CO_2 (SNK test, $p < 0.001$, Figure 4a). Moreover,
317 survival rates also dropped with increasing temperature in all grazer treatments (Figure
318 4b, Table S5). Specifically, all grazer treatments showed higher survival rates at 13.5 ºC

than at 19.9 ºC and 22.1 ºC. Comparing grazer treatments, G generally showed significantly higher survival rates (Figure 4b, Table S5).

Algal biomass in Experiment II was significantly affected by a triple interaction of CO_2, temperature and grazer (Table 2, Figure 5). Temperature and CO_2 showed no effect on grazer control treatments, which consistently revealed a ~20 % increase (from initial 7 g, 0.2g increase per day ≃ 3 % per day; 3% per day * 7 days ≃ 20 %) in algal biomass at the end of 7 days (Table S6). Algal biomass loss peaked at 19.9 ºC under ambient CO_2, while increased CO_2 led to higher algal biomass loss at 16.1 ºC (Table S6). Moreover, extreme temperatures (13.5 ºC and 22.1 ºC) consistently registered algal biomass growth, in contrast to algal loss verified in intermediate temperatures (16.1 ºC and 19.9 ºC). Under ambient CO_2 and 13.5 ºC, *Ulva rigida* growth was higher in M compared to G+M (SNK test, $p < 0.05$, Table S6).

The Dynamic Index (DI) was interactively affected by CO_2 and temperature (Table 2, Figure 6). Thus, under increased CO_2 a temperature increase from 13.5 ºC to 16.1 ºC led to an overall DI minimum, i.e. largest top-down control (SNK test, $p < 0.01$, Table S7), while at ambient CO_2 no significant effects in DI were detected (Figure 6). Under increased CO_2, further temperature increase (16.1 ºC to 19.9 ºC and 22.1 ºC) led to a consecutive increase in DI values, i.e. a reduction of top-down interaction strength (Figure 6, Table S7). Conversely, under ambient CO_2, the strongest top-down interaction (i.e. low DI, high grazer pressure) occurred at 19.9 ºC. Once again this significant trend was reverted at 22.1 ºC, and DI values indicating bottom-up control were registered (SNK test, $p < 0.001$; Figure 6, Table S7). No grazer treatment effects were observed in the DI index (Table 2).

Discussion

Our results showed that grazers' survival and consumption rates were differently affected by warming and acidification. Additionally, temperature showed immediate effects (Experiment I, two days) on survival rates, whereas acidification-related effects emerged later on (Experiment II, seven days). When co-occurring, stressor impacts were additive and independent, hence no interactive climate stressor effects were found on survival rates. Increased CO_2, i.e. hypercapnia-linked, mortality is known to occur as a consequence of prolonged animal inactivity and/or starvation due to metabolic suppression, which explains why CO_2 effects were only detected after seven days (Kurihara et al., 2008; Langenbuch and Pörtner, 2004; Spicer et al., 2007). Thus, these results strengthen the claim that warming is the strongest stressor, outweighing acidification effects on animal survival (see Wernberg et al. 2012 for a review). the relatively high mortality verified was likely caused by lower animal physiological tolerances to fixed stressor (e.g. temperature) levels in comparison to variable stressor levels (Benedetti-Cecchi et al., 2006; Vasseur et al., 2014). Contrary to natural fluctuating conditions (i.e. where stressor levels vary over time), our experimental conditions comprised of fixed stressor treatments (i.e. constant thermal and pH conditions with low intra-treatment variance), which likely contributed to increased physiological stress and mortality (Stillman, 2002).

Herbivore performance analysis also revealed striking differences between both grazers tested. Molluscs are usually considered more sensitive to warming, and especially acidification, than crustaceans (Harvey et al., 2013). In theory, due to naturally higher basal metabolism (see Peck et al., 2009), *M. palmata* may be closer to its metabolic peak (i.e. "optimal" level) than *G. umbilicalis*, thus overcoming the optimal metabolic threshold with shorter increases in stressors. Consumption rates as impacts on algal biomass were similar to those reported by Giannotti and McGlathery

(2001) for gastropod (around 0.01-0.02 grams of algae per individual and day) and by Cruz-Rivera and Hay (2001) for amphipods (around 0.004-0.01 grams of algae per individual and day) grazing on *Ulva* sp. Thus, *G. umbilicalis* showed higher impact on algae biomass, with natural temperature (16.1 ºC) and increased CO_2 amplifying algal biomass loss. Nevertheless, we found that *M. palmata* is actually a stronger grazer per biomass than *G. umbilicalis*, likely due higher metabolic rates. *G. umbilicalis* inherently higher biomass also mathematically justifies why consumption rates per biomass in mixed species treatments closely matched those reported for *G. umbilicalis*. Thus, according to our results, increases in temperature (but also in CO_2) will benifit *G. umbilicalis* by lowering *M. palmata* survival, while increasing grazing pressure on algal biomass, as predicted by the metabolic theory of ecology (MTE; Kingsolver, 2009).

Concerning abiotic effects on seaweed biomass, our results are in line with previous research, reporting no acidification effects on *U. rigida* growth (Rautenberger et al., 2015), but differ from the previously reported temperature dependence for the genus *Ulva* (Steffensen, 1976). In our study, controls (only algae, no grazers) showed similar algal biomass increase under warming and acidification conditions, in both experiments (~3 % growth per day). As such, mean algal growth rate reported here is lower than expected under perfect light intensity laboratorial conditions (20 % growth per day in Rautenberger et al., 2015). However, it is similar to results verified in field experiments, as well as for laboratorial settings mimicking subsaturating light conditions (e.g. ~5 % growth per day in Guidone et al., 2012; see also O'Connor, 2009). We suggest that the lack of algal response to climate stressors and the algal growth rates registered here were caused by comparatively reduced solar irradiation (Rautenberger et al., 2015). This reduction was a direct consequence of the greenhouse set-up housing, further amplified perhaps by the use of a 1 mm mesh on the experimental cylinders (to prevent grazers

393 from escaping). Moreover, warming-related algal growth may have been potentially
394 concealed by undetected temperature-induced algal decomposition (Schiel et al., 2004).
395 After seven days (Experiment II), algal biomass changes in treatments including
396 grazers showed the expected profile across the temperature gradient (Kingsolver, 2009;
397 Mertens et al., 2015). However, maximum algal biomass loss also depended on CO_2
398 conditions. Although interaction strength may increase with higher temperature and
399 CO_2, as shown by previous studies (Alsterberg et al., 2013; O'Connor, 2009), the
400 response patterns are indeed more complex than a direct linear antagonistic or
401 synergistic effect (Ghedini et al., 2015a). These non-linear CO_2-dependent responses to
402 temperature gradients were clearly displayed in the dynamic index profiles. As it is,
403 grazing pressure increased over increasing temperature, until survival rates decreased to
404 the point of soothing grazer pressure on macroalgae (i.e. overcoming the community
405 stability threshold), which significantly weakened macroalgae-herbivore interactions,
406 and ultimately shifted community dominance. As expected, herbivores were shown to
407 be more susceptible to changes in temperature than primary producers (López-Urrutia et
408 al., 2006). Moreover, increased CO_2 stimulated community response by accelerating the
409 interaction strength profile across warming scenarios. Thus, as predicted by Ghedini et
410 al. (2015a), the occurrence of simultaneous stressors lead to a bottom-up controlled
411 community earlier in the temperature gradient.
412 The observed warming and acidification effects were consistent in the three different
413 combinations where grazers were used, i.e. previously detected grazer identity effects
414 for other parameters were concealed in the dynamic index analyses. Thus, our results
415 did not support the claim that grazing activity by different grazer species would modify
416 the strength of top-down control on seaweeds (O'Connor 2009). Most likely our
417 election of the herbivores, without a clearly defined stronger grazer (i.e. *M. palmata* was

418 the stronger grazer per biomass, but produced a comparatively smaller impact on algal
419 biomass, and was also the most sensitive to the climate stressors) was the main cause
420 for the verified grazer homogeneity. Moreover, our experimental set-ups ran through
421 relatively short time spans (up to seven days), so extrapolating or generalizing
422 conclusions to natural systems may be inaccurate. However, it is important to note that
423 studies that were prolonged for longer periods of time (two to five weeks), generally led
424 to stronger interactions between climate change stressors (e.g. Alsterberg et al., 2013;
425 Christensen et al., 2011; Vasseur et al., 2014). Furthermore, the strength of macroalgal-
426 herbivore interactions is reported to increase from 11 to 17 days (Ghedini et al., 2015b;
427 Mertens et al., 2015; O'Connor, 2009). Therefore, we suggest that grazer identity
428 effects may still arise in the future ocean as a consequence of starkly different survival
429 and consumption rates.

430 Future climate change is expected to modify the fundamental top-down control
431 exerted by herbivore species feeding on macroalgae (Eklöf et al. 2012, this study).
432 Warming and acidification can interact and play a major role in differentially driving
433 rocky intertidal communities' structure and functioning. Extreme increases in both
434 stressors, or higher frequencies of extreme weather events (Kroeker et al., 2011), can
435 largely affect the survival rates of typical grazers. Taking advantage of lower thermal
436 sensitivity, opportunistic algal species like *U. rigida* might grow freely, disrupt
437 ecosystem equilibrium, and promote ecological shifting from top-down to bottom-up
438 regulated communities (Connell and Russell, 2010; Veteli et al., 2002). Conversely,
439 moderate increases in acidification or warming might lead to higher grazing pressure as
440 long as these stressors do not exceed metabolic grazers' thermal and acid-base limits
441 (Alsterberg et al., 2013; O'Connor, 2009), and lower algal biomass as a consequence of
442 stronger top-down control (i.e. increased interaction strength). Thus, understanding

443 whether macroalgal and herbivore responses to different climate change stressors are
444 synchronous is fundamental to assess the future strength of top-down control in marine
445 systems. We also suggest that the characteristics and composition of intertidal grazer
446 assemblages may play a relevant role in macroalgae-herbivore interactions, inducing
447 differences in grazing pressure on macroalgae, mainly due to species-specific
448 differences in survival and algal consumption rates. Longer exposures in mesocosm and
449 field experiments focusing on the combined effects of different climate stressor
450 gradients, and using diverse assemblages of herbivores and macroalgal species, are
451 needed to underpin forecasts on how macroalgae-herbivore interactions will be affected
452 by global change in the future.
453

454 **Acknowledgments**

455 The authors are thankful to all members at the Laboratory of Costal Biodiversity
456 for their assistance on species handling and maintaining the experiments. Financial
457 support was provided by project CLEF: The combined impacts of invasion and climate
458 change on coastal ecosystem functioning" (PTDC/AAC-AMB/102866/2008), an FCT
459 (Portuguese Foundation for Science and Technology) project. Research co-funded by
460 the European Regional Development Fund (ERDF) through the 'Programa Operacional
461 Factores de Competitividade' (POFC-COMPETE) within the 'Quadro de Referência
462 Estratégico Nacional' (QREN) and PEST-C/MAR/LA0015/2011.
463

References

Alsterberg, C., Eklöf, J.S., Gamfeldt, L., Havenhand, J.N., Sundbäck, K., 2013. Consumers mediate the effects of experimental ocean acidification and warming on primary producers. Proc. Natl. Acad. Sci. U. S. A. 110, 1–6. doi:10.1073/pnas.1303797110

Anderson, M., Gorley, R., Clarke, K., 2008. PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, UK.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. doi:10.1111/j.1442-9993.2001.01070.pp.x

Angilletta, M., 2009. Thermal adaptation: a theoretical and empirical synthesis. Oxford University Press, Oxford.

Aníbal, J., Rocha, C., Sprung, M., 2007. Mudflat surface morphology as a structuring agent of algae and associated macroepifauna communities: A case study in the Ria Formosa. J. Sea Res. 57, 36–46. doi:10.1016/j.seares.2006.07.002

Benedetti-Cecchi, L., Bertocci, I., Vaselli, S., Maggi, E., 2006. Temporal Variance Reverses the Impact of High Mean Intensity of Stress in Climate Change Experiments. Ecology 87, 2489–2499. doi:10.1890/0012-9658(2006)87[2489:TVRTIO]2.0.CO;2

Berlow, E., Navarrete, S., Briggs, C., 1999. Quantifying variation in the strengths of species interactions. Ecology 80, 2206–2224. doi:10.1038/18672

Byrne, M., 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages : Vulnerabilities and potential for persistence in a changing ocean. Oceanogr. Mar. Biol. An Annu. Rev. 49, 1–42.

Cacabelos, E., Olabarria, C., Viejo, R.M., Rubal, M., Veiga, P., Incera, M., Gestoso, I., Vaz-Pinto, F., Mejia, A., Engelen, A.H., Arenas, F., 2013. Ecosystem functioning impacts of the invasive seaweed *Sargassum muticum* (Fucales, Phaeophyceae). Mar. Environ. Res. 50, 1–9. doi:10.1111/jpy.12136

Christensen, A.B., Nguyen, H.D., Byrne, M., 2011. Thermotolerance and the effects of hypercapnia on the metabolic rate of the ophiuroid *Ophionereis schayeri*: Inferences for survivorship in a changing ocean. J. Exp. Mar. Bio. Ecol. 403, 31–38. doi:10.1016/j.jembe.2011.04.002

Connell, S.D., Ghedini, G., 2015. Resisting regime-shifts: The stabilising effect of compensatory processes. Trends Ecol. Evol. 30, 513–515. doi:10.1016/j.tree.2015.06.014

Connell, S.D., Kroeker, K.J., Fabricius, K.E., Kline, D.I., Russell, B.D., B, P.T.R.S., 2013. The other ocean acidification problem : CO_2 as a resource among competitors for ecosystem dominance The other ocean acidification problem : CO_2 as a resource among competitors for ecosystem dominance.

Connell, S.D., Russell, B.D., 2010. The direct effects of increasing CO_2 and temperature

on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. Proc. Biol. Sci. 277, 1409–1415. doi:10.1098/rspb.2009.2069

Cruz-Rivera, E., Hay, M.E., 2001. Macroalgal traits and the feeding and fitness of an herbivorous amphipod: The roles of selectivity, mixing, and compensation. Mar. Ecol. Prog. Ser. 218, 249–266. doi:10.3354/meps218249

Dickson, A., Millero, F., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res. 34, 1733–1743.

Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to best practices for ocean CO2 measurements. PICES Special Publication 3.

Eklöf, J.S., Alsterberg, C., Havenhand, J.N., Sundbäck, K., Wood, H.L., Gamfeldt, L., 2012. Experimental climate change weakens the insurance effect of biodiversity. Ecol. Lett. 15, 864–872. doi:10.1111/j.1461-0248.2012.01810.x

Eklöf, J.S., Havenhand, J.N., Alsterberg, C., Gamfeldt, L., 2015. Community-level effects of rapid experimental warming and consumer loss outweigh effects of rapid ocean acidification. Oikos 124, 1040–1049. doi:10.1111/oik.01544

Evans, R.G., 1948. The lethal temperatures of some common brithsh littoral molluscs. J. Anim. Ecol. 17, 165–173.

Falkenberg, L.J., Russell, B.D., Connell, S.D., 2013. Contrasting resource limitations between competing marine primary producers: implications for associated communities under enriched CO_2 and nutrient regimes. Oecologia 172, 575–583.

Falkenberg, L.J., Russell, B.D., Connell, S.D., 2013. Future herbivory: the indirect effects of enriched CO_2 may rival its direct effects. Mar. Ecol. Prog. Ser. 492, 85–95.

Gaitán-Espitia, J.D., Hancock, J.R., Padilla-Gamiño, J.L., Rivest, E.B., Blanchette, C.A., Reed, D.C., Hofmann, G.E., 2014. Interactive effects of elevated temperature and pCO_2 on early-life-history stages of the giant kelp *Macrocystis pyrifer*a. J. Exp. Mar. Bio. Ecol. 457, 51–58. doi:10.1016/j.jembe.2014.03.018

Ghedini, G., Russell, B.D., Connell, S.D., 2015a. Trophic compensation reinforces resistance: Herbivory absorbs the increasing effects of multiple disturbances. Ecol. Lett. 18, 182–187. doi:10.1111/ele.12405

Ghedini, G., Russell, B.D., Falkenberg, L.J., Connell, S.D., 2015b. Beyond spatial and temporal averages: ecological responses to extreme events may be exacerbated by local disturbances. Clim. Chang. Responses 2, 1–8. doi:10.1186/s40665-015-0014-8

Giannotti, A.L., McGlathery, K.J., 2001. Consumption of *Ulva lactuca* (chlorophyta) by the omnivorous mud snail *Ilyanassa obsoleta* (Say). J. Phycol. 37, 209–215. doi:10.1046/j.1529-8817.2001.037002209.x

Grilo, T.F., Cardoso, P.G., Dolbeth, M., Pardal, M. a., 2009. Long-term changes in amphipod population dynamics in a temperate estuary following ecosystem

restoration. Hydrobiologia 630, 91–104. doi:10.1007/s10750-009-9782-0

Guidone, M., Thornber, C.S., Vincent, E., 2012. Snail grazing facilitates growth of two morphologically similar bloom-forming *Ulva* species through different mechanisms. J. Ecol. 100, 1105–1112. doi:10.1111/j.1365-2745.2012.02002.x

Harvey, B.P., Gwynn-Jones, D., Moore, P.J., 2013. Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. Ecol. Evol. 3, 1016–1030. doi:10.1002/ece3.516

Hepburn, C.D., Pritchard, D.W., Cornwall, C.E., Mcleod, R.J., Beardall, J., Raven, J.A., Hurd, C.L., 2011. Diversity of carbon use strategies in a kelp forest community: Implications for a high CO_2 ocean. Glob. Chang. Biol. 17, 2488–2497. doi:10.1111/j.1365-2486.2011.02411.x

IPCC, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Jernakoff, P., Nielsen, J., 1997. The relative importance of amphipod and gastropod grazers in Posidonia sinuosa meadows. Aquat. Bot. 56, 183–202.

Kingsolver, J.G., 2009. The Well-Temperatured Biologist. Am. Nat. 174, 755–768. doi:10.1086/648310

Koch, M., Bowes, G., Ross, C., Zhang, X.-H., 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob. Chang. Biol. 19, 103–32. doi:10.1111/j.1365-2486.2012.02791.x

Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13, 1419–34. doi:10.1111/j.1461-0248.2010.01518.x

Kroeker, K.J., Micheli, F., Gambi, M.C., 2012. Ocean acidification causes ecosystem shifts via altered competitive interactions. Nat. Clim. Chang. 3, 156–159. doi:10.1038/nclimate1680

Kroeker, K.J., Micheli, F., Gambi, M.C., Martz, T.R., 2011. Divergent ecosystem responses within a benthic marine community to ocean acidification. Proc. Natl. Acad. Sci. U. S. A. 108, 14515–20. doi:10.1073/pnas.1107789108

Kurihara, H., Matsui, A., Furukawa, H., Hayashi, M., Ishimatsu, A., 2008. Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. J. Exp. Mar. Bio. Ecol. 367, 41–46.

Langenbuch, M., Pörtner, H.O., 2004. High sensitivity to chronically elevated CO_2 levels in a eurybathic marine sipunculid. Aquat. Toxicol. 70, 55–61. doi:10.1016/j.aquatox.2004.07.006

Larsen, B., Portner, H., Jensen, F., 1997. Extra and intracellular acid-base balance and ionic regulation in cod (*Gadus morhua*) during combined and isolated exposures to

hypercapnia and copper. Mar. Biol. 128, 337–346.

Lemos, R., Pires, H., 2004. The upwelling regime off the West Portuguese Coast, 1941–2000. Int. J. Climatol. 24, 511–524. doi:10.1002/joc.1009

Lewis, E., Wallace, D., 1998. Program developed for CO_2 system calculations.

López-Urrutia, A., San Martin, E., Harris, R.P., Irigoien, X., 2006. Scaling the metabolic balance of the oceans. Proc. Natl. Acad. Sci. U. S. A. 103, 8739–8744. doi:10.1073/pnas.0601137103

Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicx, R.M., 1973. Measurement of the apparent dissociation constantes of carbonic acid in seawater at atmospheric pressure. Limnol. Oceanogr. 18, 897–907.

Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., Pörtner, H.-O., 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6, 2313–2331. doi:10.5194/bg-6-2313-2009

Mertens, N., Russell, B., Connell, S., 2015. Escaping herbivory: ocean warming as a refuge for primary producers where consumer metabolism and consumption cannot pursue. Oecologia 173, 1223–1229. doi:10.1007/s00442-015-3438-8

Morrisey, D.J., 1988. Differences in effects of grazing by deposit-feeders *Hydrobia ulvae* (Pennant) (Gastropoda : Prosobranchia) and *Corophium arenarium* Crawford (Amphipoda) on sediment microalgal populations. II. Quantitative effects. J. Exp. Mar. Bio. Ecol. 118, 43–53.

O'Connor, M.I., 2009. Warming strengthens an herbivore-plant interaction. Ecology 90, 388–98. doi:10.1890/08-0034.1

Obenat, S., Spivak, E., Garrido, L., 2006. Life history and reproductive biology of the invasive amphipod *Melita palmata* (Amphipoda: Melitidae) in the Mar Chiquita coastal lagoon, Argentina. J. Mar. Biol. Assoc. UK 86, 1381. doi:10.1017/S002531540601441X

Pardal, M.A., Marques, J.C., Metelo, I., Lillebø, A.I., Flindt, M.R., 2002. Impact of eutrophication on amphipods *Melita palmata* and *Ampithoe valida* in the Mondego estuary., in: Pardal, M.A., Marques, J.C., Graça, M.A. (Eds.), Aquatic Ecology of the Mondego River Basin. Global Importance of Local Experience. University of Coimbra Press, Coimbra, Portugal, pp. 457–472.

Peck, L.S., Clark, M.S., Morley, S. a., Massey, A., Rossetti, H., 2009. Animal temperature limits and ecological relevance: effects of size, activity and rates of change. Funct. Ecol. 23, 248–256. doi:10.1111/j.1365-2435.2008.01537.x

Poore, A.G.B., Graba-Landry, A., Favret, M., Sheppard Brennand, H., Byrne, M., Dworjanyn, S.A., 2013. Direct and indirect effects of ocean acidification and warming on a marine plant-herbivore interaction. Oecologia 173, 111311–24. doi:10.1007/s00442-013-2683-y

620 Rautenberger, R., Fernández, P.A., Strittmatter, M., Heesch, S., Cornwall, C.E., Hurd,
621 C.L., Roleda, M.Y., 2015. Saturating light and not increased carbon dioxide under
622 ocean acidification drives photosynthesis and growth in *Ulva rigida* (Chlorophyta).
623 Ecol. Evol. 5, 874–888. doi:10.1002/ece3.1382

624 Robinson, L., Greenstreet, S., Reiss, H., Callaway, R., Craeymeersch, J., de Boois, I.,
625 Degraer, S., Ehrich, S., Fraser, H., Goffin, A., Kroncke, I., Jorgensn, L., Roberston,
626 M., Lancaster, J., 2010. Length-weight relationships of 216 North Sea benthic
627 invertebrates and fish. J. Mar. Biol. Assoc. UK 90, 95–104.
628 doi:10.1017/S0025315409991408

629 Sarazin, G., Michard, G., Prevot, F., 1999. A rapid and accurate spectroscopic method
630 for alkalinity measurements in sea water samples. Water Res. 33, 290–294.
631 doi:10.1016/S0043-1354(98)00168-7

632 Schiel, D.R., Steinbeck, J.R., Foster, M.S., 2004. Ten Years of Induced Ocean Warming
633 Causes Comprehensive Changes in Marine Benthic Communities. Ecology 85,
634 1833–1839. doi:10.1890/03-3107

635 Spicer, J.I., Raffo, A., Widdicombe, S., 2007. Influence of CO_2-related seawater
636 acidification on extracellular acid–base balance in the velvet swimming crab
637 Necora puber. Mar. Biol. 151, 1117–1125. doi:10.1007/s00227-006-0551-6

638 Steffensen, D.A., 1976. The effect of nutrient enrichment and temperature on the
639 growth in culture of *Ulva lactuca* L. Aquat. Bot. 2, 337–351.

640 Stillman, J.H., 2002. Causes and Consequences of Thermal Tolerance Limits in Rocky
641 Intertidal Porcelain Crabs, Genus *Petrolisthes*. Integr. Comp. Biol. 796, 790–796.
642 doi:10.1093/icb/42.4.790

643 Swanson, A.K., Fox, C.H., 2007. Altered kelp (Laminariales) phlorotannins and growth
644 under elevated carbon dioxide and ultraviolet-B treatments can influence
645 associated intertidal food webs. Glob. Chang. Biol. 13, 1696–1709.
646 doi:10.1111/j.1365-2486.2007.01384.x

647 Taboada, F.G., Anadón, R., 2012. Patterns of change in sea surface temperature in the
648 North Atlantic during the last three decades: beyond mean trends. Clim. Change
649 115, 419–431. doi:10.1007/s10584-012-0485-6

650 Taylor, R.B., Brown, P.J., 2006. Herbivory in the gammarid amphipod *Aora typica*:
651 relationships between consumption rates, performance and abundance across ten
652 seaweed species. Mar. Biol. 149, 455–463. doi:10.1007/s00227-006-0245-0

653 Vasseur, D.A., Delong, J.P., Gilbert, B., Greig, H.S., Harley, C.D.G., Mccann, K.S.,
654 Savage, V., Tunney, T.D., Connor, M.I.O., 2014. Increased temperature variation
655 poses a greater risk to species than climate warming. Proc. R. Soc. B. 281,
656 20132612. doi:10.1098/rspb.2013.2612

657 Veteli, T., Kuokkanen, K., Julkuken-Tiito, R., Roininen, H., Tahvanainen, J., 2002.
658 Efects of elevated CO2 and temperature on plant growth and herbivore defensive
659 chemistry. Glob. Chang. Biol. 8, 1240–1252. doi:j.1365-2486.2002.00553.x

660 Wernberg, T., Smale, D.A., Thomsen, M.S., 2012. A decade of climate change
661 experiments on marine organisms: procedures, patterns and problems. Glob.
662 Chang. Biol. 18, 1491–1498. doi:10.1111/j.1365-2486.2012.02656.x

663 Wernberg, T., Thomsen, M.S., Kotta, J., 2013. Complex plant–herbivore–predator
664 interactions in a brackish water seaweed habitat. J. Exp. Mar. Bio. Ecol. 449, 51–
665 56. doi:10.1016/j.jembe.2013.08.014

666 Wernberg, T., White, M., Vanderklift, M., 2008. Population structure of turbinid
667 gastropods on wave-exposed subtidal reefs: effects of density, body size and algae
668 on grazing behaviour. Mar. Ecol. Prog. Ser. 362, 169–179. doi:10.3354/meps07416

669 Widdicombe, S., Spicer, J.I., 2008. Predicting the impact of ocean acidification on
670 benthic biodiversity: What can animal physiology tell us? J. Exp. Mar. Bio. Ecol.
671 366, 187–197. doi:10.1016/j.jembe.2008.07.024

672 Wood, H.L., Spicer, J.I., Widdicombe, S., 2008. Ocean acidification may increase
673 calcification rates, but at a cost. Proc. Biol. Sci. 275, 1767–73.
674 doi:10.1098/rspb.2008.0343

675 Wootton, J.T., 1997. Estimates and tests of per capita interaction strength: diet,
676 abundance, and impact of intertidally foraging birds. Ecol. Monogr. 67, 45–64.

677 Wootton, J.T., Emmerson, M., 2005. Measurement of Interaction Strength in Nature.
678 Annu. Rev. Ecol. Evol. Syst. 36, 419–444.
679 doi:10.1146/annurev.ecolsys.36.091704.175535

680 Yvon-Durocher, G., Jones, J., Trimmer, M., Woodward, G., Montoya, J., 2010.
681 Warming alters the metabolic balance of ecosystems. Philos. Trans. R. Soc. Lond.
682 B. Biol. Sci. 2117–2126.

683
684

685 **Figures & Tables**

686

687 **Figure 1.** Survival rates of *G. umbilicalis* and *M. palmata* individuals present (%, mean
688 +SE) at the end of Experiment I. Data is displayed according to significant factors (non-
689 significant factor are averaged, see Table 1): a) temperature (low: 13.5 ºC, midlow: 16.5
690 ºC, midhigh: 19.9 ºC, high: 22.1 ºC), b) grazer treatments (G: *G. umbilicalis*, M: *M.*
691 *palmata*, G + M: *G. umbilicalis* + *M. palmata*. Different letters represent significant
692 differences.

693

694 **Figure 2.** Changes in mean (+ SE) algal biomass (*U. rigida* g, blot wet weight per day)
695 in the end of Experiment I. Data is displayed relative to significant factors (non-
696 interactive factors are averaged, see Table 1): a) temperature (low: 13.5 ºC, midlow:
697 16.5 ºC, midhigh: 19.9 ºC, high: 22.1 ºC), and b) CO_2 (ambient CO_2: 380 µatm,
698 increased CO_2: 830 µatm) and grazer treatments (G: *G. umbilicalis*, M: *M. palmata*, G +
699 M: *G. umbilicalis* + *M. palmata*, C: No grazers). Different letters represent significant
700 differences.

701

702 **Figure 3.** Effects on mean (+SE) herbivore consumption rates in the end of Experiment
703 I. Data is displayed relative to significant factors (non-interactive factors are averaged,
704 see Table 1): a) grazer (G: *G. umbilicalis*, M: *M. palmata*, G + M: *G. umbilicalis* + *M.*
705 *palmata*) and CO_2 treatments (ambient CO_2: 380 µatm, increased CO_2: 830 µatm), and
706 b) grazer (G: *G. umbilicalis*, M: *M. palmata*, G + M: *G. umbilicalis* + *M. palmata*) and
707 temperature treatments (low: 13.5 ºC, midlow: 16.5 ºC, midhigh: 19.9 ºC, high: 22.1
708 ºC). Results expressed as grams of algae (algae biomass) per milligrams of grazers

709 (grazer biomass) contained in the treatment, per day. Different letters represent
710 significant differences.

711

712 **Figure 4**. Survival rates of *G. umbilicalis* and *M. palmata* individuals present (%, mean
713 + SE) at the end of Experiment II. Data is displayed relative to significant factors (non-
714 interactive factors are averaged, see Table 2): a) CO_2 (ambient CO_2: 380 µatm,
715 increased CO_2: 830 µatm), and b) temperature (low: 13.5 ºC, midlow: 16.5 ºC, midhigh:
716 19.9 ºC, high: 22.1 ºC) and grazer treatments (G: *G. umbilicalis,* M: *M. palmata,* G + M:
717 *G.umbilicalis* + *M. palmata*). Different letters represent significant differences.

718

719 **Figure 5.** Effects of temperature (low: 13.5 ºC, midlow: 16.5 ºC, midhigh: 19.9 ºC,
720 high: 22.1 ºC) and grazer treatments (G: *G. umbilicalis,* M: *M. palmata,* G + M:
721 *G.umbilicalis* + *M. palmata*, C: No grazers) on mean (± SE) algal growth (*U. lactuca* g,
722 blot wet weight) at a) ambient CO_2 (380 µatm) and b) increased CO_2 (830 µatm) in the
723 end of Experiment II. Different letters represent significant differences.

724

725 **Figure 6**. Effects of temperature (low: 13.5 ºC, midlow: 16.5 ºC, midhigh: 19.9 ºC,
726 high: 22.1 ºC) and CO_2 treatments (ambient CO_2: 380 µatm, increased CO_2: 830 µatm)
727 on the Dynamic Index (DI, mean ± SE) as a strength measure of the herbivore-
728 macroalgae interaction (Experiment II). Data is displayed according to significant
729 factors (non-significant factor is averaged, see Table 2). Different letters and numbers
730 represent significant differences.

731

732

733 **Table 1.** Summary of PERMANOVA for the effects of temperature (T, 4 levels: low, midlow, midhigh, high), CO_2 (2 levels: ambient and
734 increased), and grazer (G, 3 levels: *G. umbilicalis*, *M. palmata*, *G. umbilicalis* + *M. palmata*) treatments on grazer survival and consumption rates
735 for Experiment I. DF: Degrees of freedom, MS: Mean squares. Significant values are in bold ($p < 0.05$).
736

	Grazer survival rate				*Algal biomass*				*Consumption rate*			
Source of variation	DF	MS	F	*P* (perm)	DF	MS	F	*P* (perm)	DF	MS	F	*P* (perm)
CO_2	1	7133.5	14.911	**0.0004**	1	0.1700	11.852	**0.0018**	1	0.0002	3.7689	0.0596
T	3	13368	27.944	**0.0002**	3	0.0410	2.8572	**0.0470**	3	0.0001	1.2986	0.2862
G	2	3059.4	6.3952	**0.0052**	3	0.8490	59.190	**0.0002**	2	0.0102	241.45	**0.0002**
CO_2 x T	3	538.84	1.1263	0.3466	3	0.0285	1.9904	0.1318	3	0.0001	2.4633	0.0762
CO_2 x G	2	397.38	0.8307	0.4386	3	0.0497	3.4651	**0.0256**	2	0.0001	1.5419	0.2124
T x G	6	1315.6	2.7500	**0.0242**	9	0.0226	1.5745	0.1414	6	0.0001	2.0158	0.0814
CO_2 x T x G	6	145.32	0.3038	0.9324	9	0.0110	0.7697	0.6466	6	0.0001	3.2616	**0.0106**
Residuals	48	478.40			64	0.0143			48	0.0000		
Total	71				95				71			

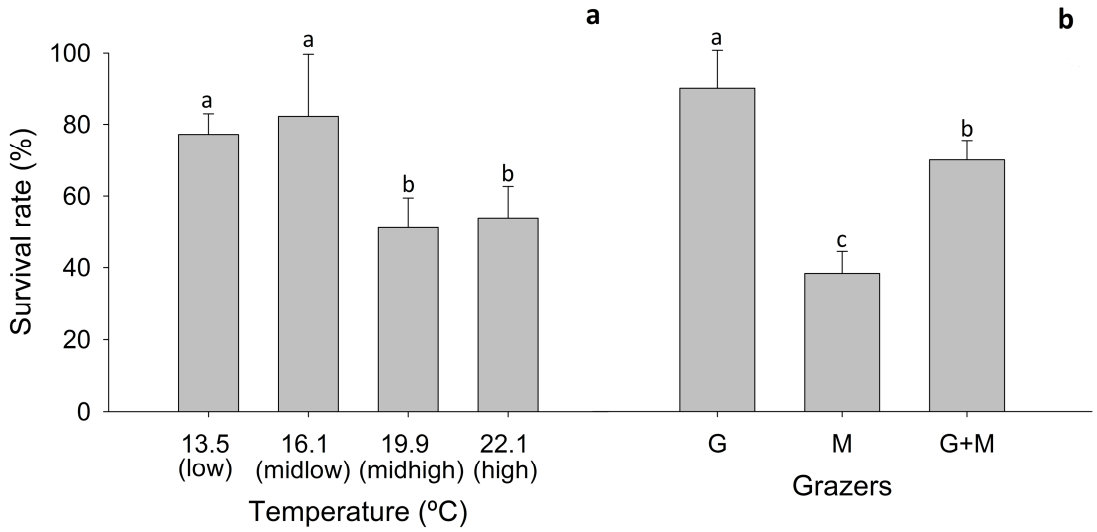
737

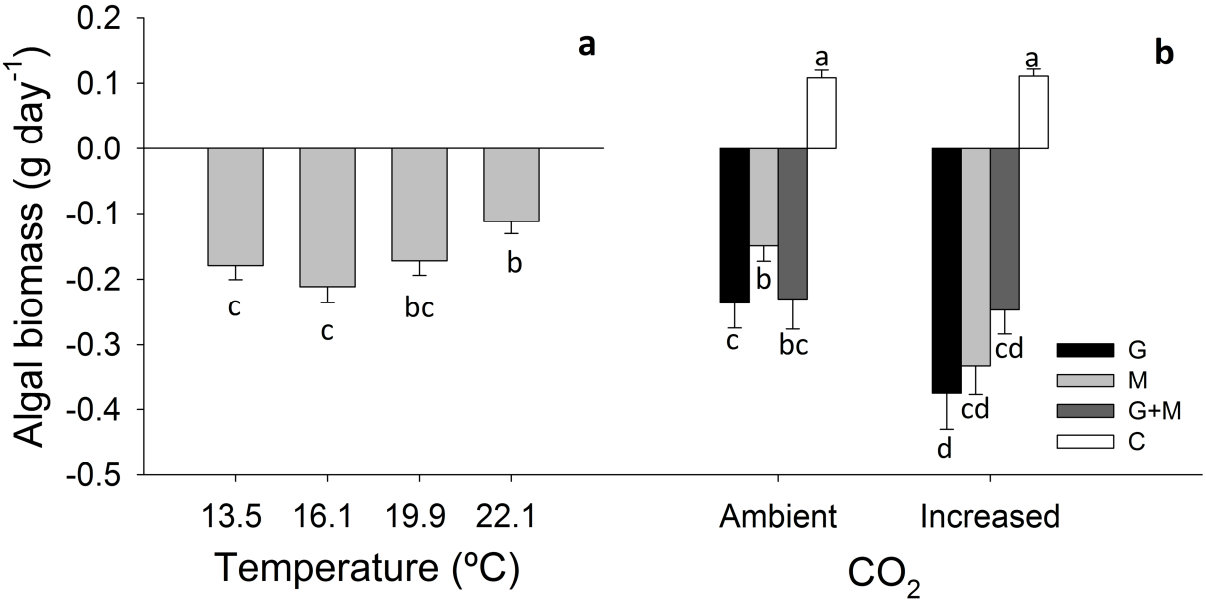
738 **Table 2.** Summary of PERMANOVA for the effects of temperature (T, 4 levels: low, midlow, midhigh, high), CO_2 (2 levels: ambient and
739 increased), and grazer (3 levels: *G. umbilicalis, M. palmata, G. umbilicalis* + *M. palmata*) treatments on grazer survival rate, algal growth and
740 Dynamic Index for Experiment II. DF: Degrees of freedom, MS: Mean squares. Significant values are in bold ($p < 0.05$).

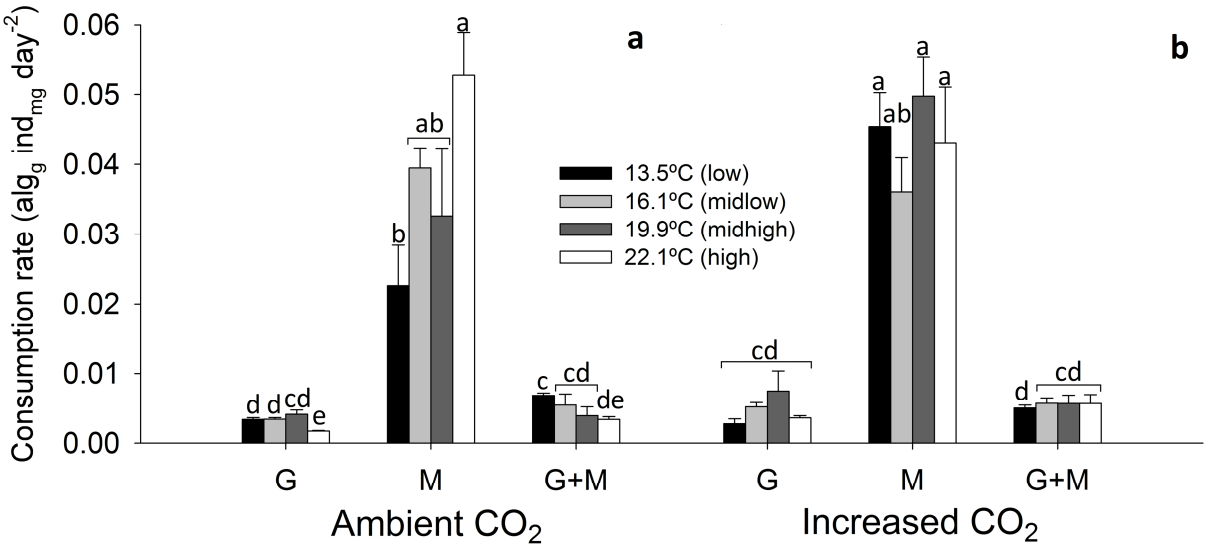
741

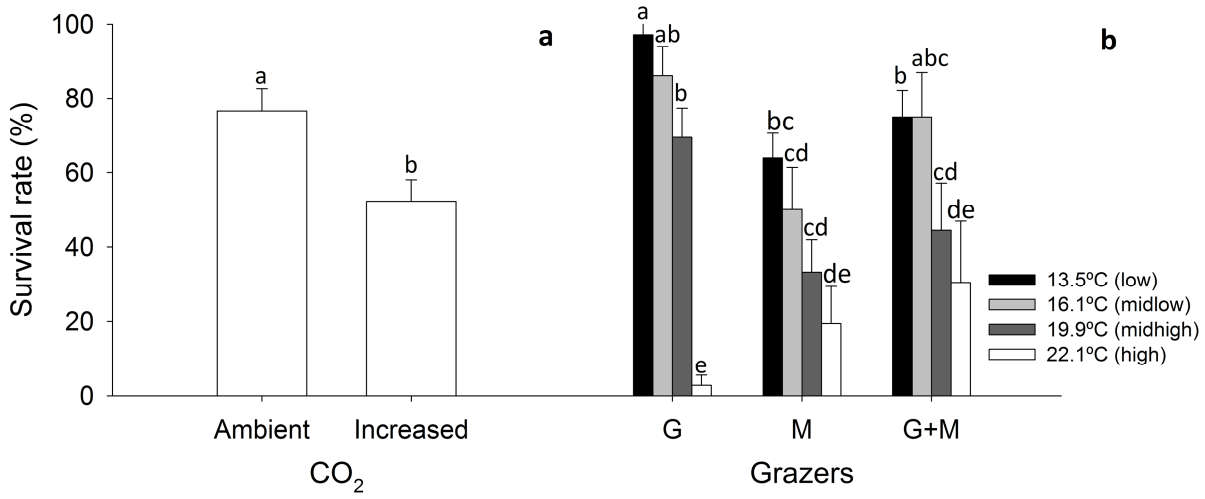
	Grazer survival rate				*Algal biomass*				*Dynamic Index*			
Source of variation	DF	MS	F	*P* (perm)	DF	MS	F	*P* (perm)	DF	MS	F	*P* (perm)
CO_2	1	22.22	0.0465	0.8202	1	0.0010	0.1223	0.7204	1	0.3155	8.0122	**0.0042**
T	3	4544.4	9.5116	**0.0002**	3	0.4468	52.9910	**0.0002**	3	0.8288	21.046	**0.0002**
G	2	16289	34.093	**0.0002**	3	0.2097	24.8660	**0.0002**	2	0.0346	0.8784	0.4376
CO_2 x T	3	277.78	0.5814	0.6246	3	0.2306	27.3470	**0.0002**	3	0.8202	20.826	**0.0002**
CO_2 x G	2	72.222	0.1511	0.8544	3	0.0316	3.7510	**0.0154**	2	0.0499	1.2681	0.2886
T x G	6	833.33	1.7442	0.1294	9	0.0546	6.4809	**0.0002**	6	0.0325	0.8250	0.5646
CO_2 x T x G	6	394.44	0.8256	0.547	9	0.0447	5.2981	**0.0006**	6	0.0445	1.1306	0.3576
Residuals	48	477.78			64	0.0084			48	0.0394		
Total	71				95				71			

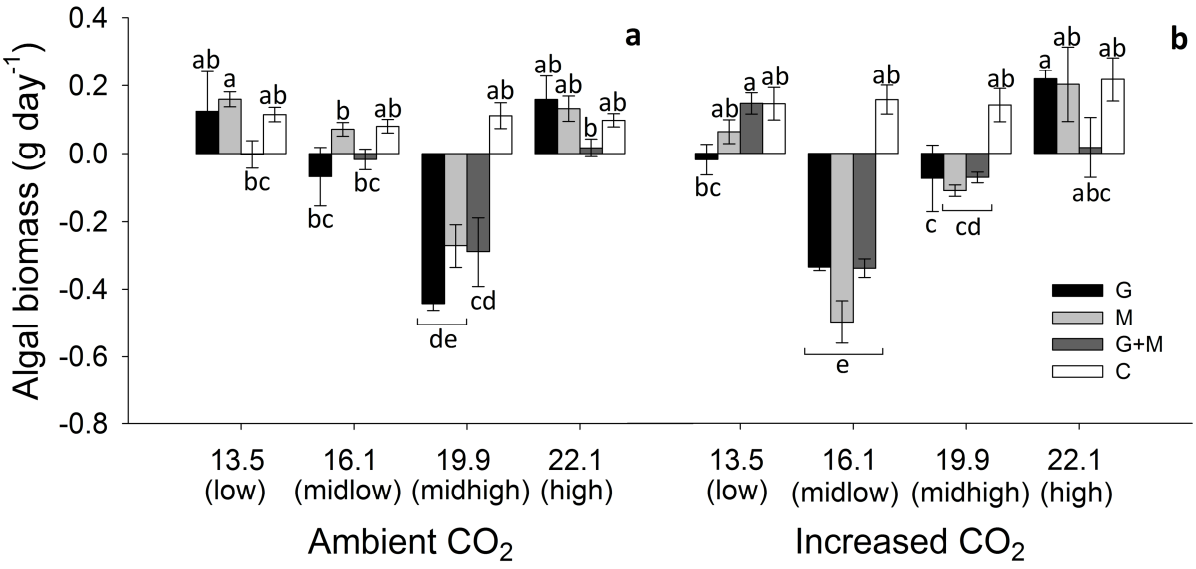
742

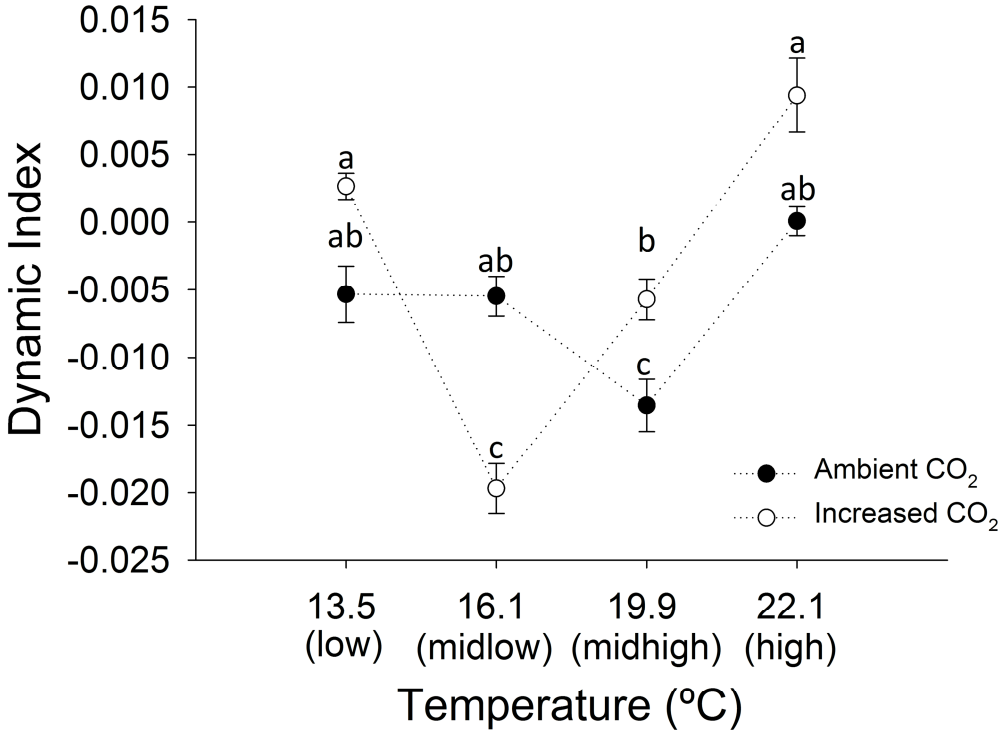












Highlights

- Warming and acidification elicited negative effects in grazer's survival rates.
- Macroalgae-herbivore interaction strength was shaped by temperature gradient.
- Acidification shifts thermal optimal metabolic threshold to lower temperatures.
- Grazer identity may modulate macroalgae-herbivore interactions.